Influence of hydrodynamics on the growth kinetics of glass-adhering *Pseudomonas putida* cells through a parallel plate flow chamber

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(Received 28 May 2013; accepted 1 September 2013; published online 11 September 2013)

The objective of this work was to investigate the influence of hydrodynamics on the growth kinetics of surface-adhering *Pseudomonas putida* cells. The results showed in particular that under non substrate-limiting conditions, the early step of bacterial apparent growth rate is lower than those measured with suspended cells. Contrary to previously cited authors which explain this behavior to the different adhesive properties of the "daughter"-cells (which makes more probable the detachment of these daughter-cells), in our experimental conditions, that explanation does not hold and we show a clear dependence of growth kinetics with flow conditions, due to the formation of boundary layer concentration at low Reynolds number. These results revealed that using Monod law in the modeling of biofilm growth in fixed-biomass processes should be performed with care. © *2013 AIP Publishing LLC*.
[http://dx.doi.org/10.1063/1.4821244]

I. INTRODUCTION

Modeling biofilm growth in porous media is a key issue in many industrial processes (wastewater biofiltration, soil remediation). As stated by Devinny and Ramesh,[1] we are far from having operational models for such processes. This is mainly due to the complex task accounting for the collective behavior of living cells in biofilms.

Most models dealing with biofilm growth, either based on upscaling techniques or directly written on a global scale, use the well-known Monod kinetics which is usually applied for the growth of suspended bacteria. This is not so straightforward, even for the early steps of biofilm development. For instance, as observed in the literature, some research works have revealed phenotype changes for suspended bacterial cells after their initial adhesion, in relation with cell-to-cell signaling through chemical communication (*quorum sensing*).[2–4] Investigations at a more local scale are thus required in order to gather a better knowledge on the local behavior of bacterial cells attached on a solid substrate and in order to write suitable models accounting for their growth kinetics.

Microfluidic systems have been widely used in order to reproduce life conditions of individual cells or microbial communities and thus in order to perform local studies on microorganisms or biological cells submitted to various physical, chemical, or biochemical stresses. For instance, and among existing works, Preira *et al.*[5] designed a microfluidic device to

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measure the rheological properties of cells submitted to a flow-induced shear stress. Yazdi and Ardenaki[6] investigated in a micro channel the influence of fluid flow on dynamics of motile microorganisms and their aggregation. They showed that vortical structures promoted cell aggregation and triggered biofilm streamers formation. Furthermore, they show that the bacterial collection in such a vortical flow is strongly pronounced for high motile bacteria. Sun *et al.*[7,8] developed micro-devices to study the cell response and migration under the action of an electrical field. One of their devices was designed to mimic *in vivo* electric field distribution of a wound. The aim was to study the coupling of an electric field with the pharmacodynamics of potential wound-healing drugs and its consequence on the wound-healing rate. In another study, they developed a device to study the electrostatic response of lung cancer cells in alveoli-like 3D scaffolds. Through comparison with cells examined in 2D gelatin-coated and bare substrates, they observed clear differences in cell morphology, directedness, and migration speed under electrostatic field between the 2D and 3D environment.

All the studies presented above did not address the role played by hydrodynamics on growth kinetics of surface-associated bacteria. To the best of our knowledge, few studies have been indeed performed to investigate specifically the early stage of growth of initially adhering bacteria or cells on solid surfaces[9–11] in terms of growth kinetics and its coupling with the local hydrodynamics.

Though hydrodynamics is a determining factor in many biological, physical, or chemical mechanisms. In particular mass transfer, which affects directly nutrient availability towards bacterial cells, and then their growth rate,[12] is strongly dependent on flow conditions.[13,14] Yet, the couplings between these local mechanisms are kinetically controlled (time-dependent)[15] and sensitive to initial conditions of surface colonization.

This work fits within this scientific issue. A parallel plate flow chamber was specifically developed to follow *in situ* the dynamic behavior of bacterial cells under hydrodynamic constraints. The study is interested in the kinetics of growth for bacteria initially adhering to the lower plate of the flow chamber which is considered as the solid substratum. The investigations are inclined in particular towards the effects of shear stress on bacterial growth kinetics, under controlled nutrient fluid flow at low Reynolds number.

II. MATERIAL AND METHODS

A. Bacterial system and growth medium

A Pseudomonas putida 6521 strain was selected as bacterial model for this study. This aerobic rod-shaped bacterium (diameter 0.5–1.0 μm, length 1.5–4.0 μm) is able to degrade a wide range of organic chemicals, making it a good candidate for ecological engineering studies. The negative charge and hydrophilic character of its cell-surface were previously established.[16]

The preparation of growth medium was based upon Luria Bertani (LB) technique but with modified concentrations for the chemical components. A mixture containing 5 g yeast extract, 5 g pure sodium chloride, and 950 Ml deionized water was homogenized and its pH adjusted to 7 with diluted sodium hydroxide. Then its volume was adjusted to 1 l, autoclaved at 120 °C during 20 min, and finally cooled in room temperature under laminar flow hood. In the following sections, this obtained growth medium will be called "modified LB." Compared to the classical composition of the LB medium, the casein peptone was replaced by phenol. This modified medium was used because the present work is included in a larger project dealing with organic compounds (containing phenol molecules).[17]

B. Biomass preparation

Aliquots of *P. putida* suspensions stored at −20 °C in 20% glycerol were used to isolate colonies from Petri agar plates,[18] which were then used to inoculate 200 ml of modified LB growth medium described above. To limit bacterial contamination, *P. putida* suspensions were then supplemented with phenol at 20 mg/l. Bacteria were grown at 27 °C overnight in a rotary tumbler.

Growth kinetics of *P. putida* were first determined for liquid suspensions containing free living cells, by inoculating 200 μl of previously grown bacterial suspension in 150 ml of modified LB growth medium under rotational shaking at 200 rpm. Periodic samplings were performed as described below: a sample of isolate was incubated at 27 °C under magnetic agitation. After each interval time of 1 h during the incubation, a 1 ml bacterial solution was sampled from the growing culture and its absorbance was measured. The exponential rate of growth was then measured to be $\mu = 0.013\,\text{min}^{-1}$. The incubation was stopped at the beginning of the stationary phase, which occurred at time t = 15 hours and a 0.5 ml aliquot of bacterial suspension was collected for experimental tests. The same was done for all experimental tests, leading to the same biological and physical-chemical properties of the biomass for each experiment. Thereafter, a preliminary preparation of dilution and cell washing was applied to the 0.5 ml sample as follow:

– 20 times dilution in 9.5 ml sterile sodium chloride 0.15 mM,
– centrifugation 10 min at 8000 rpm,
– separating bacteria from nutritive liquid medium,
– suspending bacteria in 10 ml sterile sodium chloride 0.15 mM,

This procedure was performed in duplicate in order to ensure a successful biomass preparation.

C. The parallel plate flow chamber

Mass transport through the substratum surface of a microfluidic flow chamber is linked to fluid flow.[14,19] However, exceeding a critical limit (Re > 1400) may prevent cell adhesion, as this phenomenon is dependent on shear stress distribution (Gaver and Kute[20]). Furthermore, Gaver and Kute showed theoretically that even for smaller cells, the stress, force, and torque can be significantly greater than those predicted for flow in a cell-free system. Thus for the investigation of bacterial growth kinetics on surface, hydrodynamics of the flowing medium through parallel plates must be well controlled (especially if one wants to favor adhesion and prevent cell detachment). In particular, a uniform distribution of wall shear stress on the substratum of cell adhesion is a mandatory condition. For this reason, the different kinds of parallel plate flow chambers are evaluated in terms of the size area of substratum surface where the flow velocity profile is established with a wide range of flow rates;[21] this is the main criterion for evaluating the designing of flow chamber. For instance, the length of establishment of the flow velocity depends strongly on the shape of the inlet of the flow chamber. It is also shown that wall effects depend on relative dimensions of the transversal section, in particular on the ratio height/width.[22] A low value of this ratio is then necessary to ensure a 2D flow and thus a uniform distribution of wall shear stress.

Our parallel plat flow chamber was specifically developed upon these criteria. It is composed of 3 elements as shown in Fig. 1:

(i) The upper plate made in PMMA with following dimensions: length 75 mm, width 25 mm, thickness 13 mm. This piece was specifically machined to form the inlet, outlet and a hole

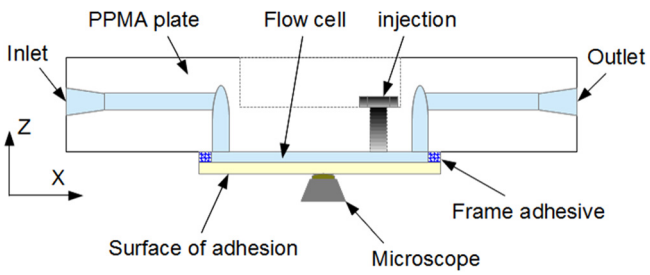

FIG. 1. Description of the flow chamber.

for the injection of bacterial suspension. The inlet and outlet are connected, respectively, upstream to a 100 ml syringe and downstream to a disposal tank.

(ii) An adhesive hollow frame (*ABgene, Thermo Scientific, Illkirch, France*) which internal dimensions are length L = 28 mm, width w = 17 mm and thickness h = 0.25 mm, is placed between the upper PMMA plate and the lower plate, therefore delimiting a parallelepiped and ensuring waterproofness.

(iii) The lower plate used as solid substratum for cell adhesion and being of two types. The first is a glass sheet (*Menzel-Gläser, Braunschweig, Germany*) with 32 mm length, 22 mm width, and 0.15 mm thickness; it is used for initial bacterial growth and biofilm development. The second is a clear vinyl plate (*Electron Microscopy Sciences, Hatfield, PA, USA*) with 75 mm length, 25 mm width, and 0.5 mm thickness; supporting high flow rates without deformation, it is used for studying the kinetics of cell detachment induced by shear stress, permitting evaluation of the cell adhesion strength.

Hydrodynamics within the flow chamber was characterized by means of the Particle Tracking Velocimetry technique. Results showed that the flow velocity followed a parabolic profile between the two parallel plates (Fig. 2). Measurement along longitudinal direction showed also that the velocity profiles were auto-similar (Fig. 3), indicating that the flow was established in the area of observation[23] and that no deformation of the plates occurred. Measurements in the transverse direction (data not shown) proved also that the flow was of 2D profile; this is justified by ratio h/w $\ll$ 1, where the height h = 0.25 mm and width w = 17 mm, allowing to disregard wall effects in area of observation. The wall shear stress *Tw* is thus

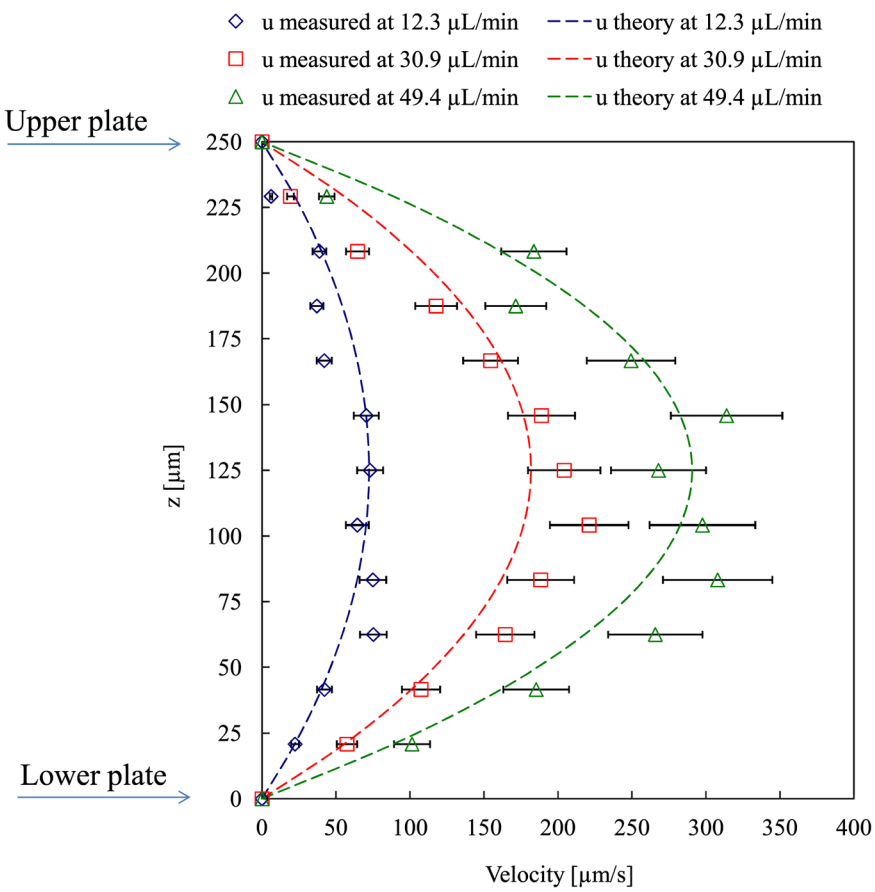

FIG. 2. Velocity profile between the two parallel plates at three flow rates. Experimental data (colored symbols) coupled with theoretical curves (dotted lines).

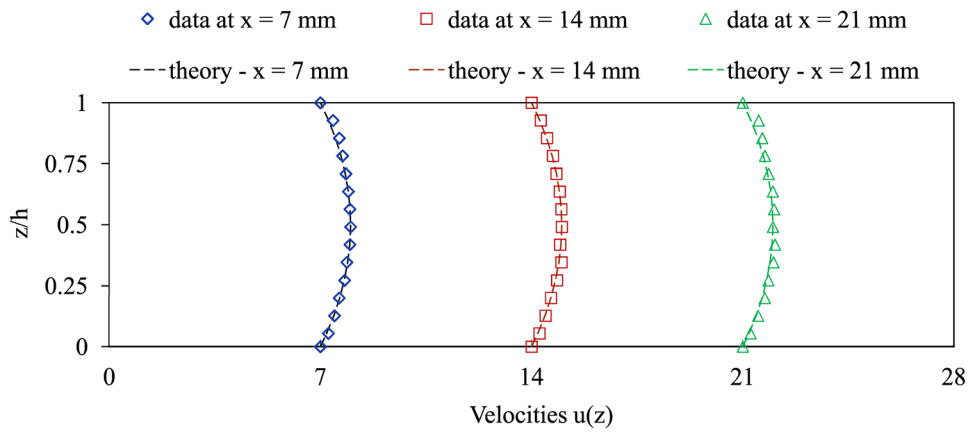

FIG. 3. Variation of local velocity profile along x-direction. Data obtained for 3 points of a streamline x1 = 7; x2 = 14 and x3 = 21 mm. h = 250 μm. Experimental data (colored symbols) coupled with theoretical curves (dotted lines). Flow rate- = 100 μl/min.

uniform in the measurement area and is a function of the flow rate Q, and dimensions of flow chamber cross-section (h, w), as expressed by

$$T_w = \mu \frac{du}{dz}\bigg|_{wall} = \frac{6\mu Q}{h^2 w}, \tag{1}$$

u = u(z) is the local longitudinal velocity at altitude z, the wall (surface of adhesion or lower plate) being considered as reference z (at lower plate) = 0; μ (Pa.s) is the dynamic viscosity.

D. Protocol implementation for bacterial adhesion and growth

All elements of the flow system were cleaned in sterile conditions (under a laminar flow hood) using the following procedure:

(i) Glass supports were immersed in a sulfochromic solution ($K2Cr_2O_7$, H_2SO_4) for 3 h and completely rinsed in sterilized deionized water.
(ii) Clear vinyl plates were absolutely cleaned by 95% ethanol and rinsed in sterilized deionized water.
(iii) The 100 ml syringe, the PMMA plate and tubular connections were assembled, cleaned by circulation of 95% ethanol and subsequently rinsed with sterile deionized water.

After cleaning procedure, a lower plate was sealed to the PMMA plate with an adhesive hollow frame, forming the flow chamber assembled with tube connections. These elements together were filled with sterile growth medium and solidly placed in an inverted microscope (*IX70, Olympus*). Fluid circulation was maintained by a syringe pump (*Lambda Vit-Fit, Zurich, Switzerland*).

Under static conditions, a 100 μl bacterial suspension was injected in the flow chamber through its injection hole, leading to the initiation of cell sedimentation phase. After an initial adhesion of several bacteria, cells injection was stopped and a low flow rate of 100 μl/min was established to remove all non-adhering cells until t = 10 min. Then flow was stopped, ensuring static conditions. At an initial contact time Tc of 30 min, flow of nutritive medium was applied at a fixed flow rate. Image acquisition of the same observed area was performed at Tc = 30 min and then every 20 min.

Measurement of growth kinetics was performed directly by visual counting of cells adhering to the solid support. With this procedure, the enumeration error was estimated to be less than 4%. At first, we used the Image processing toolbox of Matlab to perform an automated counting of cells contained in each image. This classical method, mainly based upon binary

and threshold functions, works correctly for raw images corresponding to the early stage of surface colonization (low-concentrated surfaces until $t = 200$ min). However, gradually as time passes and cells multiply, they make aggregates. It becomes then difficult to separate individual cells in order to enumerate them without getting erroneous information: the method appeared very sensitive to the choice of threshold coefficient, which makes an automatic enumeration impossible.

Thus, all results presented here were obtained from careful manual enumeration of bacterial cells adhered in the area of observation. To proceed, the picture was printed with A4 size format. The total area of picture was then divided into smaller sub-areas to reduce counting errors. The total number of adhered cells was then the sum of the number of cells of sub-areas. The counting was performed in triplicate to assess the results.

E. Assessment of the experimental conditions

Two conditions were required, to assess our experimental results: (i) neither bacterial deposition (ii) nor detachment during the growth process.

1. Bacterial deposition

The absence of bacterial deposition was maintained by the use of a non-contaminated incoming culture medium. Furthermore, the output effluent were not re-circulated but rather evacuated to a disposal downstream tank.

2. Bacterial detachment

The hydrodynamic effects on growth kinetics were investigated using glass substratum as lower plates, permitting to ensure that no subsequent detachment was induced by shear stress after initial adhesion. Indeed, preliminary measurements conducted with glass plates showed that cell adhesion was too strong (data not shown), as it did not permit an accurate evaluation of the kinetics of cell detachment from this surface at our low shear stress conditions. That is why complementary experiments were performed with clear vinyl plates as solid substrata. The use of vinyl plates permitted to evaluate the time scales of bacterial detachment as functions of shear stress intensity, using a classical experimental approach.[24,25] These experiments consisted in submitting surface-adhering bacteria to increasing flow-induced shear stresses and to investigate the effect of environmental conditions on their surface-adhesion strength. Obtaining controlled high flow rates required replacement of the syringe injection system by a volumetric pump (*Eheim Compact 600*) connected to a 1 l upstream tank. A flowmeter permitted to control the equilibrium flow rate established in the flow chamber. Results (data not shown) showed that for this less adhesive solid substratum, for an ionic strength of 15 mM and a moderate initial contact time (15 min), cell detachment occurred only if the shear stress was above 10 Pa. When the contact time increased, the critical shear stress increased (up to 60 Pa to observe detachment with a contact time equal to 30 min (Fig. 4). The same was observed when the ionic strength increased (data not shown).

The absence of cell detachment during growth processes was then ensured using glass plate as a solid substratum, and ensuring a contact time of about 30 min as stated in Sec. II D. Cells that were not adhering irreversibly to the solid support after this contact time were previously eliminated before initiating the growth process by applying a relatively low flow rate during the process.

These conditions permitted to obtain reproducible results for the measurement of growth parameters and ensure that no detachment occurred during the experiments in our operating conditions (Table I).

III. RESULTS

The growth rate of adhering bacterial cells was calculated successfully, owing to the measurement of cell multiplication under non-detachment conditions. Fig. 5 shows the image of

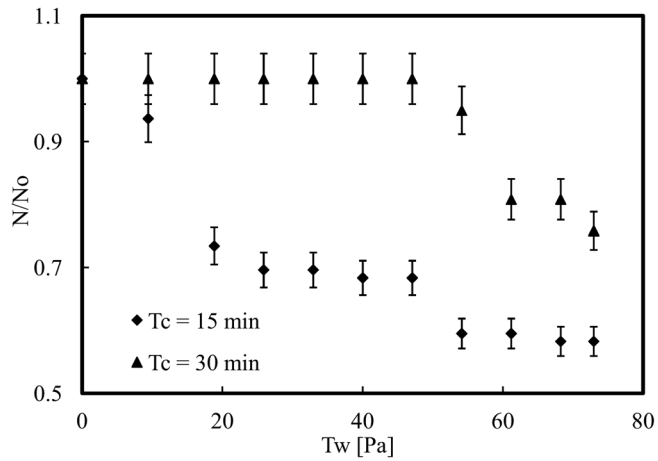

FIG. 4. Evolution of the relative number (N/No) of *P. putida* cells remaining attached under increasing wall shear stress (N: number of instantaneous attached cells; No = N(t = 0)) for 2 different initial contact times; clear vinyl plate as solid substratum; sodium chloride 15 mM as liquid medium; T = 24 ± 1 °C.

early surface-associated cells growing in a same observed area from 0.5 h to 5 h 30 min after cell adhesion. After an initial lag phase, bacterial growth kinetics was found to follow an exponential law (Eq. (2)) as shown in Fig. 6 for two hydrodynamic conditions

$$N(t) = N_0 \times \exp(\mu t). \tag{2}$$

N(t) is the number of adhering cells at time t, N_0 being its initial value and μ the growth rate expressed in min^{-1}. Four different hydrodynamic conditions were tested. The associated measured growth rates are presented in Table II. Fig. 7 shows that increasing the Reynolds number increased the growth rate of adhered bacterial cells. The experimental data were best fitted with power law, with the following form:

$$\mu = k.\text{Re}^{\alpha}. \tag{3}$$

The measured growth rate μ was shown to be increased with increasing Reynolds number up to the value of growth rate measured for suspended (non-adhering) cells in similar conditions of growth. Only the flow configuration was different: free rotational flow for the suspended cells and tangential sheared flow for adhered cells.

IV. DISCUSSION

In this work, growth kinetics of initially glass-adhered *P. putida* cells was investigated. Attached bacterial cells, submitted to the flow of a growth medium in a specially designed flow chamber, presented a growth rate always lower than those measured for planktonic cells. However, an increase in Reynolds number (by increasing flow rate) in the flow chamber leads to an increasing bacterial growth rate, approaching the one obtained for the *P. putida* planktonic cells ($0.013\ \text{min}^{-1}$). These results demonstrate clearly that the growth of adhering cells is

TABLE I. Hydraulic conditions during growth of the initially surface-adhered bacteria. Q, Re, and Tw are, respectively, the flow rate, Reynolds number, and wall shear stress; "min" and "max" mean, respectively, the minimum and maximum value of these hydraulic parameters.

Q (ml/min)		Re (-)		Tw (mPa)	
Min	Max	Min	Max	Min	Max
0.10	10.63	0.09	10	9	1000

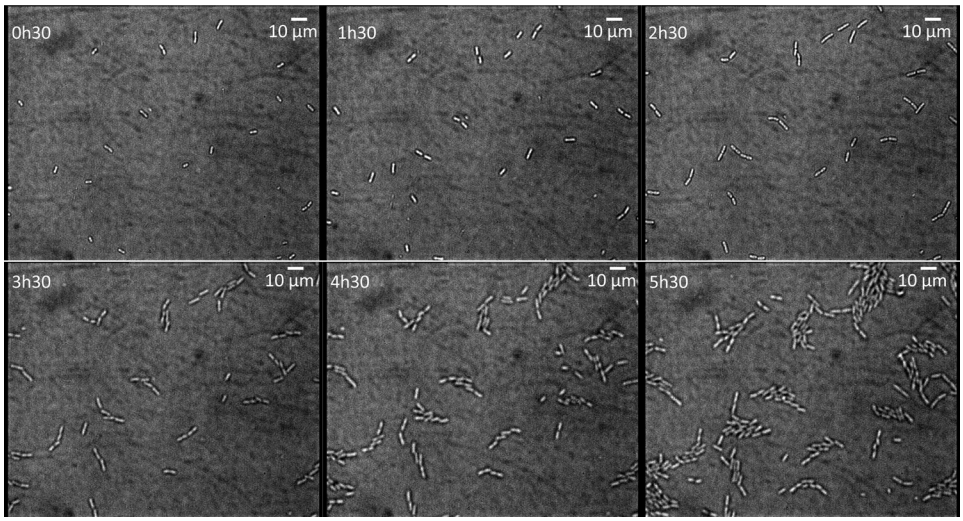

FIG. 5. Visualization of the growth of *P. putida* cells adhered on glass support at Re = 0.8, Tw = 76.8 mPa; bacterial colonies were *in situ* followed from 30 min to 5 h 30 min; T = 24 ± 1 °C.

limited by nutrient availability. The maximum growth rate measured in our experiments can be considered as the optimal growth rate since fresh medium is continuously supplied to the cells with an upstream syringe.

Our results are in agreement with Barton *et al.*[9] who investigated the growth rate of bacterial colonies on polymeric surfaces under laminar flow in a vertical flow chamber but at a single Reynolds number. They observed lower bacterial growth rates with attached cells than with suspended cells. These authors interpreted their data as an apparent growth rate. They determined, using surface free energy measurements that daughter-cells and initially adhering cells had different surface properties, with the consequence of a higher detachment rate that decreased the apparent growth rate at a flow rate corresponding to Re = 1.5, without considering other flow rates.

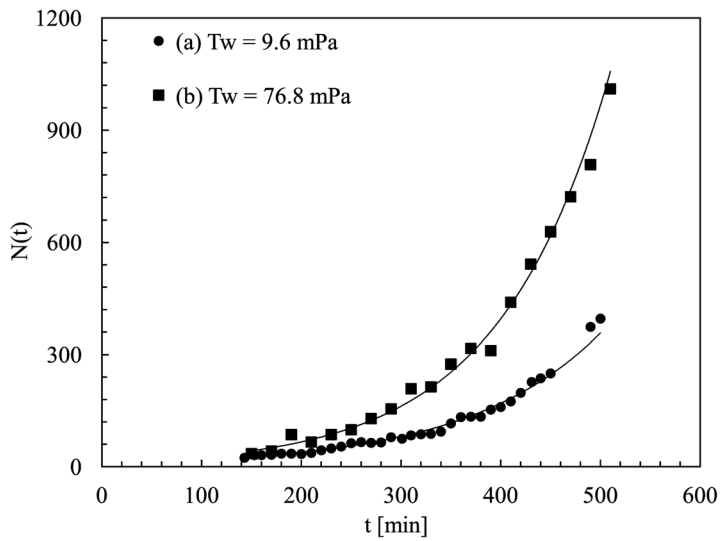

FIG. 6. Growth kinetics of *P. putida* cells on glass surface under flow shear stress (a) Re = 0.1 and Tw = 9.6 mPa, circle = experimental data, solid line = data fit with exponential law: growth rate $\mu = 0.0074\,\text{min}^{-1}$, $R^2 = 0.99$; (b) Re = 0.8 and Tw = 76.8 mPa, square = experimental data, solid line = data fit with exponential law: growth rate $\mu = 0.0089\,\text{min}^{-1}$ and $R^2 = 0.98$.

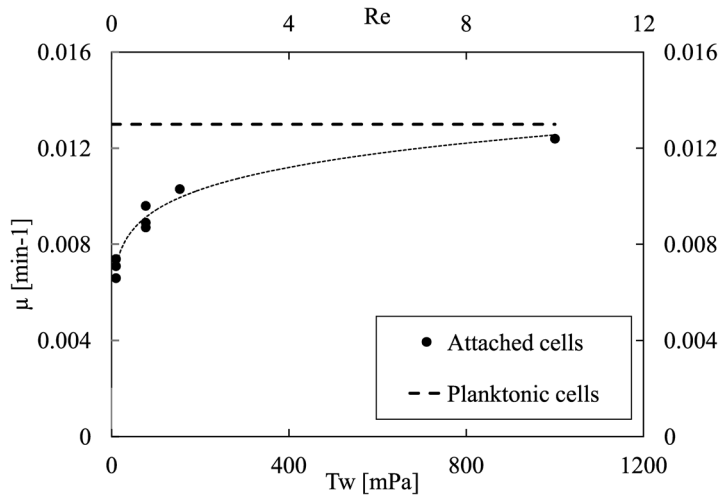

FIG. 7. Variation of surface-associated growth rate of *P. putida* cells adhering on glass surface versus wall shear stress Tw and Reynolds number Re, at T = 24 ± 1 °C; solid line = adjustment with power law (k = 0.005, $\alpha = 0.125$ and $R^2 = 0.96$). The dashed-line corresponds to the growth rate constant determined with suspended cells. For Tw = 1000 mPa, the results is an average from the *in situ* following of 10 bacterial colonies, which is an alternative for repeating this assay (1000 mPa).

Gottenbos *et al.*[10] investigated the surface growth of initially adhering organisms on different materials as a function of their water contact angles. Their work revealed that an increasing water contact angle of cellular surface leads to a reduction of bacterial generation time, meaning a rise in growth rate, without further interpretation of the involved mechanisms.

Recently, Gödeke *et al.*[11] investigated the bacterial surface-associated growth in a flow chamber. They revealed several transcriptional changes during the earlier stage of cells surface colonization, and especially the expression changes in functional genes coding for transport and conversion of carbohydrates, amino-acid transport and metabolism, lipid metabolism, gluconate catabolism, and also phage genes regulation.

None of these works addressed the problem of cell division under variable hydrodynamic conditions which has been shown to be potentially a controlling factor in biofilm structure and compaction.[26] Indeed, hydrodynamics may affect some important metabolism processes such as mass transfer from the bulk medium towards the vicinity or inside the cells.

In the light of these works, our dataset is novel since detachment processes cannot be invoked to explain the flow-dependency of the growth rate of adhering cells. Indeed, if we consider cell division in suspended bacterial growth system, the maximal growth rate was $0.013\,\text{min}^{-1}$, corresponding to a generation time of 53 min, which is in agreement with published data for *P. putida* system.[23] Such generation time is much longer than the initial cell-surface contact time (Tc = 30 min) necessary to obtain a condition of zero cell detachment during growth experiments, even when combining low ionic strength and higher shear stresses. This means that cell division time is long enough to allow daughter-cells to get irreversibly attached to the solid surface.

TABLE II. Growth rate constants of glass-adhering *P. putida* cells determined during the exponential growth phase, at increasing wall shear stress, Tw, and Reynolds number, Re.

Tw (mPa)	Re	N(t = 0)	μ (min^{-1})
9.6	0.1	41	0.007
76.8	0.8	47	0.009
153.4	1.6	43	0.010
1000	10.42	43	0.012

Furthermore, cell deposition on the surface of adhesion is unlikely during experiments, since the flow chamber was continuously supplied with non-contaminated culture medium. In this condition, detached cells would be carried out upstream without re-deposition owing to the flow-induced drag force.

Thus, the surface colonization of the measurement area is here only due to cells duplication of initially adhering bacteria. As only the flow rate was changed from one experiment to another, the observed increase of the growth parameter must however be explained by considering the modification in flow conditions. Furthermore, this trend on the growth parameter values cannot be associated to the effect of the flow on the detachment/deposition rate of the bacteria and consequently to a modification of the balance between the cells duplication and the net flux of bacteria cells inside and outside the measurement area.

To explain our measurements, one of the processes that can be invoked is nutrient limitation at low shear due to diffusion mechanisms. Indeed, substrate degradation may lead to the formation of a boundary layer concentration around attached bacterial cells. Therefore, global mass transfer would depend both on the development of this very local boundary layer concentration and nutrient penetration through membrane bacterial cells. Results presented in Fig. 7 are thus consistent with diffusion-controlled processes in *quasi* static conditions (low Reynolds number). The dependence as a power of the Reynolds number is also consistent with boundary layer theory. Similarly, limitation by oxygen concentration (final electron acceptor) can be invoked, although the feeding solution was continuously aerated by stirring. The maximum solubility of O_2 in this medium and at the operating temperature is quite low (7 mg/l) and may be limiting. Whilst this observation was surprising because the global concentration of nutrients in culture medium are far in excess compared with the initially adhering cells, this is the most plausible explanation of the observed results.

In the field of engineering, this result seems to indicate that caution must be taken when using Monod law to analyze growth kinetics of attached biomass. For example, in the modeling of activated sludge processes, investigation of growth kinetics can be performed by the use of results obtained for suspended-cell cultures. This can be justified by similarities of hydrodynamic conditions between activated sludge and suspended growth cultures. Our flow chamber with fixed-cell cultures presented laminar flow conditions with low stresses. Mass transfer process through bacterial membrane is dictated by the existence of a thin boundary layer substrate concentration, even if this concentration is always constant outside the boundary layer.

If mass transfer and nutrient limitation are considered when dealing with biofilm modeling, one must account for the different scale at which these phenomena occur.[17] If most models integrate the biofilm resistance to transfer, phenomena occurring at the bacterial scale are often discarded: it is assumed that Monod kinetics is valid and that the local nutrient concentration is the effective concentration seen by the cells. This can lead to confusions in some models found in literature. The problem is quite complicated. For instance, to overcome the lack of knowledge on the couplings between biological processes and hydrodynamic processes at the microscopic scale, many engineering models are directly written at macroscopic scale using a set of convection-dispersion-reaction equations. Biofilm growth is then modeled through a closure law, whose structure is postulated *a priori* and often based on Monod kinetics. For instance, Kildsgaard and Engegaard[27] and Brovelli *et al.*[15] made remarkable investigations to study the development and spreading of biofilms in 2D porous media from the early stage. These works were based upon an idea developed by Zysset *et al.*:[28] the biofilm growth rate was modeled by a Monod law (with constant parameters not dependent on flow conditions) modulated by a limitation function in order to take into account mass transfer and biofilm activity limitation as the biofilm becomes thicker. This function depends on the volume fraction ϕ of the biofilm, approaching 1 at minimal ϕ, and 0 and at maximal ϕ. The critical limit ($\phi = 0$) corresponds to the earlier phase of bacterial colonization of individual cells and at this stage, growth kinetics appears then to be non-flow dependent: this is incompatible with our results.

V. CONCLUSION

This work investigated the influence of hydrodynamics on the growth kinetics of glass-adhering *P. putida* cells through a parallel plate flow chamber. Experiments were conducted at different Reynolds numbers (shear stress) and under conditions of non cell detachment/re-attachment.

Our results showed that the growth rate of adhering cells was strongly controlled by shear stress imposed to the cells, and tended to increase up to the growth rate measured for planktonic (suspended) cells system. In our opinion, even if a cellular response (through genetic expression, metabolism change.) could be put forward, a simplest and consistent explanation is a limitation of nutrient availability due to the existence of boundary layer concentration at local scale.

These results show that the Monod law seems is not adequate to study growth kinetics for attached cells and biofilm, and that, a general closure law integrating effects of hydrodynamics as well as biofilm structure and physiological states should be sought.

This general closure law could be written directly at the biofilm scale. It should integrate a set of representative parameters accounting for the coupling between the biofilm structure and its evolution with time (for instance, the biofilm volume fraction, the EPS volume fraction, the biofilm specific surface, etc.) and the operating conditions (for instance, the initial shear stress on the surface free of cells, etc.). Such a law could be more convenient to address all the complexity of the biofilm growth process. The exact structure of this law and the choice of the relevant parameters are beyond the scope of this paper: that is not an easy task and should require a great deal of experimental efforts.

Another strategy would consist in working at the bacteria scale and then deduce a general closure law using upscaling techniques. Compared to the first approach, a refined knowledge of the relevant processes accounting for the cells behavior at the bacteria scale is then required. That raises a number of scientific issues such as, as it has been shown here, a better knowledge on the coupling between hydrodynamics, transport phenomena at the bacterial scale, and bacterial physiological state itself.

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